Exercise increases the proportion of fat utilization during short-term consumption of a high-fat diet[1–3]

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ABSTRACT

Background: Increases in energy substrate oxidation occur at different rates after an increase in either fat or carbohydrate intake. Adaptations to increased fat intake are relatively slow and are influenced by activity level.

Objective: We tested the hypothesis that increased levels of daily activity, as influenced by added exercise, would have a graded effect on the rate of compensatory adjustment to a short-term high-fat diet.

Design: Daily total energy expenditure and macronutrient oxidation were measured at 3 physical activity levels (PALs) by using a whole-room indirect calorimeter in 10 adult women as they transitioned from a 1-d low-fat (30% of energy) control diet to a 4-d high-fat (50% of energy) diet. The 3 PALs (1.4, 1.6, and 1.8) were provided daily by increases in bicycle ergometer exercise time.

Results: An increase in physical activity led to a greater increase in the nonprotein respiratory exchange ratio (-0.047 ± 0.02, -0.064 ± 0.02, and -0.071 ± 0.02; $P < 0.0001$) and 24-h fat oxidation (113 ± 24, 125 ± 19, and 147 ± 20 g/d; $P < 0.0001$) for PALs of 1.4, 1.6, and 1.8, respectively, after the transition from the low-fat control diet to the high-fat diet. Random-effects analysis found a significant ($P = 0.003$) relation between PAL and the compensatory fat oxidation response to a high-fat diet.

Conclusions: Amounts of exercise consistent with the Institute of Medicine's recommendations reduce the time required to match fat oxidation to a change in the percentage of fat in the diet. Because short-term consumption of high-fat diets is thought to contribute to excess fat accumulation, regular exercise should be protective and should help maintain a healthy body composition. *Am J Clin Nutr* 2007;85:109–16.

KEY WORDS Obesity, metabolism, high-fat diet, fat oxidation, exercise, indirect calorimetry

INTRODUCTION

Obesity, often defined by body mass index (BMI; in kg/m^2), is more correctly defined as a state of excess body fat. This condition develops through an accumulation of fat during periods of positive fat balance. Accumulation of excess body fat has been causally linked to diets high in fat in several epidemiologic studies (1, 2). In addition, a simple decrease in the contribution of fat to a diet characteristically leads to a loss in body weight (3–5), which is enhanced with contributions from voluntary caloric restriction (1, 6). The obesogenic effects of dietary fat have been attributed to its high energy density and palatability, which lead to passive overconsumption (7, 8); its high efficiency of dietary fat storage, which leads to a more positive energy balance (9, 10); and a weaker metabolic feedback system of appetite control for fat, which leads to greater energy consumption and fat accumulation (9).

In contrast, carbohydrate overfeeding studies found the presence of homeostatic controls that quickly increase carbohydrate oxidation in response to overfeeding and that subsequently balance dietary carbohydrate intake with oxidation (11). Fat overfeeding studies indicated that no similar balancing mechanism exists for fat (12–16), but eucaloric diet studies in which the percentage of energy intake from fat was increased showed a shift in fat oxidation that eventually balanced intake with oxidation (17–19). However, these dietary fat and carbohydrate feeding studies found that the homeostatic adjustments to changes in macronutrient intakes seem to differ between carbohydrate and fats. Whereas carbohydrate oxidation quickly adjusts to balance changes in dietary carbohydrates (11), fat oxidation adjusts much more slowly to acute changes in dietary fat (16, 17, 19, 20). Thus, an acute exposure to a high-fat diet results in a positive fat balance for several days after this dietary change but before fat balance occurs (21).

Because of the slow adaptive response to changes in dietary fat intake, positive fat balance is readily apparent during an acute change to a high-fat diet (19, 20). More recently, however, it was shown that the adjustment in fat oxidation is accelerated by an increased level of exercise in men (21). On the basis of these data, we hypothesized that a similar response would be seen in women and that a threshold effect on fat oxidation would be seen with levels of exercise in excess of a physical activity level (PAL) of 1.7 [PAL = total energy expenditure (TEE) divided by resting metabolic rate (RMR)] during consumption of an isocaloric high-fat diet. To test this hypothesis, we examined the effect of 3 PALs on fat oxidation in sedentary women after transition from

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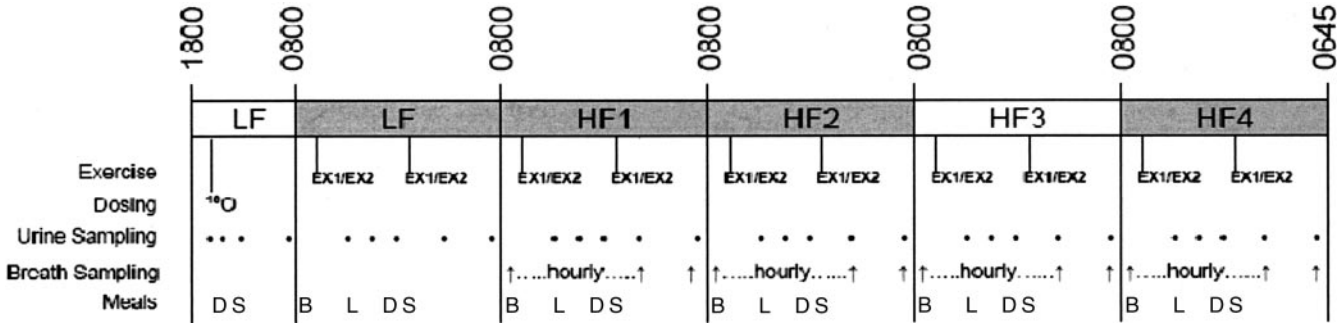

FIGURE 1. Study timeline for all treatments used in this crossover experimental design. Subjects changed from a low-fat (LF) diet to a high-fat (HF) diet at all meals, including breakfast (B), lunch (L), dinner (D), and snack (S). HF1–4, days 1–4 of the HF diet. Subjects were in a whole-room indirect calorimetry chamber on the LF diet day and on HF1, HF2, and HF4.

a diet with 30% of energy from fat to a diet with 50% of energy from fat.

SUBJECTS AND METHODS

Study volunteers

Fourteen volunteers started the protocol, but only 10 were used in the current analysis. Two women dropped out because of pregnancy or claustrophobia, respectively; in addition, the data for 2 participants were lost because of equipment failures, and those participants were unable to return for completion of study.

Ten sedentary female volunteers were recruited by using print advertising. After providing informed consent, they underwent a comprehensive laboratory and physical examination. For this randomized crossover study, the volunteers were selected for sex, age 18–39 y, BMI 20–30, moderately sedentary lifestyle (<3 h/wk of low-to-moderate-intensity exercise and no vigorous exercise), regular menstrual cycle, absence of metabolic disease (eg, thyroidism, hyperlipidemia, or diabetes), and a absence of cardiac abnormalities. Regular menstrual cycle is defined as one that has a readily recognizable beginning and end and a periodicity of 21 to 35 d for at least the previous 6 mo. Volunteers participating in the study were allowed to take birth control pills. All volunteers were admitted for their inpatient visits during the follicular phase of their menstrual cycle.

The subjects were informed of the study protocol and associated risks before giving their written consent. This study protocol, consent form, and advertising were approved by the University of Wisconsin Institutional Review Board.

Protocol

Participation in this randomized crossover study included preliminary outpatient screening and 3 inpatient stays of 5 d and 6 nights each (**Figure 1**) within a metabolic chamber at the University of Wisconsin-Madison General Clinical Research Center (GCRC). Preliminary testing included a graded exercise peak stress test (peak oxygen consumption: $\dot{V}O_{2peak}$), which was completed at the University of Wisconsin Sports Medicine Clinic by using an electronically braked cycle ergometer (Lode, Groningen, Netherlands) and a metabolic cart (CPZ/D; Medical Graphics, St Paul, MN). Active data were collected for ≈5 min while the volunteers pedaled with no resistance and while they breathed through a 2-way Hans-Rudolph valve. When the subjects reached a steady state under conditions of no resistance, the graded $\dot{V}O_{2peak}$ test was initiated at 20 W and continued under 15-W stepwise increases for 2-min intervals until the volunteer was exhausted. The $\dot{V}O_{2peak}$ value was accepted as valid only when the respiratory exchange ratio (RER) increased to >1.0 and a $\dot{V}O_2$ plateau was seen.

During the outpatient visit and after an overnight fast, RMR (in kcal/d) and respiratory exchange ratio [ie, the ratio of carbon dioxide production ($\dot{V}CO_2$) to $\dot{V}O_2$] were measured by using a ventilated-hood metabolic system (Deltatrac I; SensorMedics, VIASYS Healthcare, Yorba Linda, CA). During calibration of the Deltatrac I to medical gas (96.0% O_2 and 4.0% CO_2), participants rested for 30 min in the supine position. They were then instructed to remain motionless without sleeping while the $\dot{V}CO_2$ and $\dot{V}O_2$ measurements were taken. The 3 levels of 24-h total energy intake for the 3 treatments [sedentary (SED), 1 h exercise/d (EX1), and 2 h exercise/d (EX2)] were designed to equal predicted energy expenditure (EE) and were 1.4×, 1.6×, and 1.8× RMR, respectively.

Once all preliminary testing was completed, the randomized inpatient visits began (Figure 1). To match expenditure and thus minimize changes in energy balance, the 3 inpatient visits differed only in EE (duration of exercise) and caloric intake. For 3 d before each inpatient visit, participants were provided with packaged foods and microwavable meals for all meals and snacks, as described below. Participants were asked not to participate in any vigorous physical activity for the day before the day of admission to the GCRC and to do nothing more than activities of daily living on the day of admission.

After fasting for ≥4 h, participants were admitted to the GCRC in the evening (1800) to begin the 6-night, 5-day inpatient visit. They were asked to give a urine sample for pregnancy testing and for the measurement of baseline $H_2{}^{18}O$ (18). At 1900 each day during each inpatient visit, participants were also given a dose of $H_2{}^{18}O$ (0.025 g · kg body weight) for measurement of total body water (TBW) and body composition from urinary $H_2{}^{18}O$. Immediately afterward, dinner was served. Participants were asked to sleep beginning at 2300 on each night of the inpatient visits. Moreover, daytime sleeping was discouraged, and subjects were awakened when appropriate.

The diet containing 30% of energy from fat [ie, the low-fat (LF) diet] began with the lead-in diet and continued through the evening admission and the first full day in the metabolic chamber. Each morning, the volunteers were awakened and exited the chamber at 0645; they reentered the chamber at 0800. While they were out of the chamber, RMR was measured and then they were

allowed 30–45 min for personal hygiene. EE for the out-of-chamber time (75 min) was estimated by using $\dot{V}CO_2$ and $\dot{V}O_2$ data from the daily RMR measurement (30 min) and the equivalent data measured during the first 45 min after reentry into the chamber each morning (before exercise and minimizing thermic effect of meals).

After the subjects reentered the chamber, breakfast was given at 0830, and prescribed exercise took place each day roughly between 1000 and 1100 and again between 2100 and 2200. During each day of the inpatient visit, research meals were eaten at 1200 and 1900, and a snack was given at 2200 or at the completion of the exercise period.

The provision of high-fat (HF) meals, described below, began on day 2 and lasted the next 4 d until visit completion. During each visit, day 3 of the HF diet was spent without chamber data collection to allow for a psychological break from the confinement. Day 3 was designed to be identical to the rest of the days, by including diet, activity, and sampling. Discharge occurred ≈0800 on the sixth morning in the GCRC.

Exercise

The treatment design was based on varying EE between treatments as a result of longer durations of same-intensity exercise. In the EX1 and EX2 conditions, exercise on a cycle ergometer was performed twice daily, ending at 1100 and again at 2200, for a total expenditure of 150 and 300 kcal, respectively, during all exercise. Because individual durations of cycling to reach the stipulated exercising expenditure varied, start times were adjusted for the prescription of individually different durations of cycling. Exercise duration varied between conditions and was ≈0 (SED), 60 (EX1; 150 kcal), and 120 (EX2; 300 kcal) min of daily cycling, of which half was performed in the morning and half in the evening. Most participants ($n = 7$) needed slightly more than two 1-h bouts of cycling in EX2, and therefore their exercise period lasted beyond 1100 and 2200. Exercise intensity was 45% $\dot{V}O_{2peak}$.

A 24-h EE (1.4 × RMR) reflective of a sedentary condition was determined by previous observational experience of participants from multiple cohorts under sedentary conditions while they spent whole days in the metabolic chamber. The EX2 PAL (ie, 1.8) was selected on the basis of the study of Smith et al (21), and the EX1 PAL (ie, 1.6) was chosen as an intermediate level of physical activity.

To determine the duration of exercise, the energy cost of cycling at 45% $\dot{V}O_{2peak}$ was estimated from the linear equation between $\dot{V}O_2$ and work (measured in W) that occurred during the preliminary graded cycle ergometer $\dot{V}O_{2peak}$ test. Relative $\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$) at 45% $\dot{V}O_{2peak}$ was used to calculate a rate of EE (at kcal/min) at that intensity (W), which was used in combination with RMR to estimate minutes of cycling needed to increase 24-h TEE for each treatment.

Meals

For 3 d before each inpatient visit, volunteers consumed a packaged weight-maintenance diet with 30% of energy from fat. The diet included packaged food and meal supplements (Boost Plus and Boost Bars; Mead Johnson, Evansville, IN) to reach the desired energy levels and state of satiety. Participants were asked to eat only to satiety and to provide a written record of what they consumed. The average intake during this time was 2025 ± 358 kcal/d (59 ± 1% of energy from carbohydrate, 26 ± 1% from fat, and 15 ± 0.3% from protein). Individually, the treatments averaged 1976, 1991, and 2108 kcal/d for SED, EX1, and EX2, respectively, and they did not differ significantly ($P = 0.74$).

On admission to the GCRC, the participants were provided with research meals from the kitchens of the University of Wisconsin Hospitals and Clinics. All LF meals were designed to provide 30% of energy from fat, 55% from carbohydrate, and 15% from protein, and all HF meals were designed to provide 50% of energy from fat, 35% from carbohydrate, and 15% from protein. Energy intake was provided in a roughly isocaloric manner by predicting it as RMR × PAL for SED (RMR × 1.4), EX1 (RMR × 1.6), and EX2 (RMR × 1.8). In matching predicted 24-h EE including SED, EX1, and EX2, energy intake was modified by proportionately increasing portion sizes of the diet. Fatty acid profiles of all diets were held constant with equal proportions of saturated, monounsaturated, and polyunsaturated fatty acids (1:1:1).

Meals (Figure 1) were given at 0830 (breakfast), 1200 (lunch), and 1900 (dinner); a snack was given at 2200 (snack) each day of the visit. Individual daily meals consisted of 25%, 25%, 40%, and 10% of daily caloric needs for breakfast, lunch, dinner, and snack, respectively. The morning meal (all days) consisted of a mixed macronutrient dairy-based shake. The shakes were served at room temperature, and the empty container was rinsed twice with hot water, which was consumed by the volunteers while in the metabolic chamber. Participants were required to eat all meals completely. If total consumption was not possible, any remaining meal was weighed, described, and accounted for.

Respiratory chamber

The respiratory chamber at GCRC was designed to be similar to the chamber in the Department of Human Biology at Maastricht University in Maastricht, Netherlands (22), and its specifications and diagnostics are described elsewhere (23). Briefly, this one-room chamber has a 12-m^3 internal volume with 2 outlets. One outlet is an airlock pass-through that is used for passing food, drink, and biological samples between inside and outside of chamber. The second is the chamber entryway that can be opened opened from either side.

The chamber temperature is kept at 23 °C by using a chilled water and dehumidification system that keeps temperature and humidity constant. The air inlet and outlet of the chamber allow outside air to be drawn through the chamber at a rate of ≈80 L/min, which produces a slight negative pressure inside the chamber of −5 to −7 mm of mercury. The composition of the air is measured by using carbon dioxide (Hartman and Braun Uras-4) and oxygen (Magnos-6) gas analyzers (Applied Automation, Bartlesville, OK). Data acquisition software (LABVIEW version 2; National Instruments, Austin, TX) and an NB-T10-10 data acquisition interface were used to acquire gas analyzer outputs. Values for $\dot{V}O_2$, $\dot{V}CO_2$, RER, and EE were obtained by using a spreadsheet macro program (EXCEL, version 2002; Microsoft, Redmond, WA) designed for the chamber.

The calibration of the chamber was confirmed by conducting methanol burns (22). Anhydrous methanol (99.8% pure; Fisher Scientific, Pittsburgh, PA) was burned for 8–16 h, and the mean ± SD percentage error in recovery was −0.30 ± 0.25% for carbon dioxide and 1.0 ± 1.6% for oxygen. For most of the study, the ratio of average measured $\dot{V}CO_2$ produced to $\dot{V}O_2$ consumed (ie, RER) was 0.660, whereas the theoretical ratio is 0.667. On

occasion, the methanol recoveries were less than theoretical, and this difference was traced to a flow mismatch through the detectors. During this period, the RER averaged 0.636. The results from the subjects studied during this period were adjusted for the calibration error.

Calculation of energy and macronutrient oxidation

EE and substrate oxidations were calculated by using the equations of Jequier and Schutz (24) for $\dot{V}CO_2$ produced, $\dot{V}O_2$ consumed, and urinary nitrogen. After adjustment for the difference between day and night protein oxidation (urinary nitrogen excretion), they were separated, and EE and substrate oxidations were calculated on the basis of day and night and were summed for 24 h measures. Thus, macronutrients oxidized were converted to nutrients, calculated in grams oxidized in 24 h, and EE was converted to TEE, calculated in kilocalories directly from 24-h total nonprotein $\dot{V}CO_2$ and $\dot{V}O_2$ values and their subsequent nonprotein RER (NPRER) values.

Sample collection and analysis

For the measurement of ^{18}O, 1 mL urine was allowed to equilibrate with carbon dioxide at 25 °C for 48 h. ^{18}O enrichment was measured by using continuous-flow isotope ratio mass spectrometry, and body water was calculated by dilution on the assumption that the oxygen dilution space was 1.007 × TBW (25). Each sample was run on 2 separate days, and each run included 3 injections. The average SD of the runs was 0.19 per mil, which is propagated to a CV of 1% on the TBW determination. Fat-free mass was calculated as TBW divided by 0.73.

Urinary nitrogen samples were collected at intervals of ≈4 h; additional collections were made when necessary. Aliquots (15 mL) of all urine produced in the chamber were saved and acidified with 250 mg citric acid (Acros Organics/Fisher, Chicago, IL) to prevent the volatilization of the nitrogen compounds. The volume of all urine produced while subjects were in the chamber was recorded. Samples were kept at −5 °C until their dilution for analysis of nitrogen content. When necessary, 1% (by vol) of each void collected between the scheduled collections was combined to obtain a representative sample of urine output for that period. The urine aliquots were then diluted 1-to-100 in triple-distilled water for analysis. Nitrogen analysis was performed by using a chemiluminiscence nitrogen analyzer (Antek 9000N; Antek Instruments Inc, Houston, TX). Nitrogen concentration was measured in parts per million against a calibration curve with known concentrations of urea (0–100 ppm).

Statistical analysis

All statistical analyses were completed by using SAS software (version 8.02; SAS Institute Inc, Cary, NC). Each subject served as her own control in a randomized crossover study design. We defined an outlier as a point that lies outside of the mean ± 3 SDs. No outliers were identified. PROC MIXED was used for the repeated-measures analysis to analyze the effects of exercise with time (SED, EX1, and EX2) on the primary dependent variables. All multiple *t* tests were Bonferroni corrected. All values are presented as mean ± SEM unless otherwise indicated, and $P \leq 0.05$ was considered significant.

TABLE 1
Subject characteristics[1]

Characteristic	Value
Age (y)	25 ± 3.5
Weight (kg)	73.6 ± 16.9
BMI (kg/m^2)	25.9 ± 4.0
Lean mass (kg)	47.3 ± 11.2
Fat mass (kg)	22.7 ± 16.9
Body fat (%)	33.7 ± 5.7
RMR (kcal/d)	1408 ± 220
$\dot{V}O_{2peak}$ (mL · kg^{-1} · min^{-1})	32.5 ± 7.7
Fasting insulin (pmol/L)	48 ± 32.6
Fasting glucose (mmol/L)	4.9 ± 0.2
Triacylglycerol (mmol/L)	1.0 ± 0.5
Thyrotropin (μIU/mL)	1.1 ± 0.4
EX1 cycling time (min)	33 ± 3.4
EX2 cycling time (min)	66 ± 6.9

[1] All values are $\bar{x}$ ± SD; $n = 10$. RMR, resting metabolic rate; $\dot{V}O_{2peak}$, peak volume of oxygen consumed; EX1, 1 h exercise/d; EX2, 2 h exerise/d.

RESULTS

Subjects

Ten healthy female subjects completed this study (**Table 1**). All of the subjects described themselves as sedentary (<3 h/wk of low-to-moderate-intensity exercise and no vigorous exercise). Their measured $\dot{V}O_{2peak}$ was 32.5 ± 7.68 mL · kg^{-1} · min^{-1}. The participants' BMIs ranged from reference (ie, 20–24.9) to overweight (ie, 25–29.9), but blood lipids, glucose, and insulin were in the normal range.

Energy budget

Energy intake

Energy intake was recorded for each day, and no differences were seen between the intakes on the days of the HF diet, and thus values were averaged for all HF diet days. Therefore, mean 24-h energy intakes with the LF and HF diets [combined mean for HF diet days 1, 2, and 4 (HF1, HF2, and HF4)] in all treatments were 2025 ± 96 and 2084 ± 102 kcal/d (SED), 2310 ± 110 and 2363 ± 116 kca/d (EX1), and 2547 ± 129 and 2619 ± 134 kcal/d (EX2), respectively. When LF and HF diets are combined, the average 24-h energy intake for SED, EX1, and EX2 (2069 ± 48, 2350 ± 55, and 2601 ± 64 kcal/d, respectively) showed a significant ($P < 0.0001$) main effect of exercise by design (ie, EX2>EX1>SED). No significant main effect of time ($P = 0.89$) or exercise × time interaction ($P > 0.99$) was found. Individually, the ratio of energy intake to RMR (energy intake level) was 1.47 ± 0.01 (SED), 1.67 ± 0.003 (EX1), and 1.85 ± 0.01 (EX2). These energy intakes had small daily variability by design. However, the energy intake slightly exceeded our design of 1.4×, 1.6×, and 1.8× RMR.

Energy expenditure

The women cycled for an average of 0 ± 0, 33 ± 3, and 66 ± 7 min/d for the SED, EX1, and EX2 conditions, respectively. By design, TEE showed a main effect of exercise ($P < 0.0001$) and averaged 1963 ± 99, 2152 ± 103, 2368 ± 136 kcal/d for SED, EX1, and EX2, respectively. These values showed no interaction ($P = 0.99$) or main effect of time ($P = 0.98$). PAL increased as exercise volume increased from SED (1.40 ± 0.01) to EX1

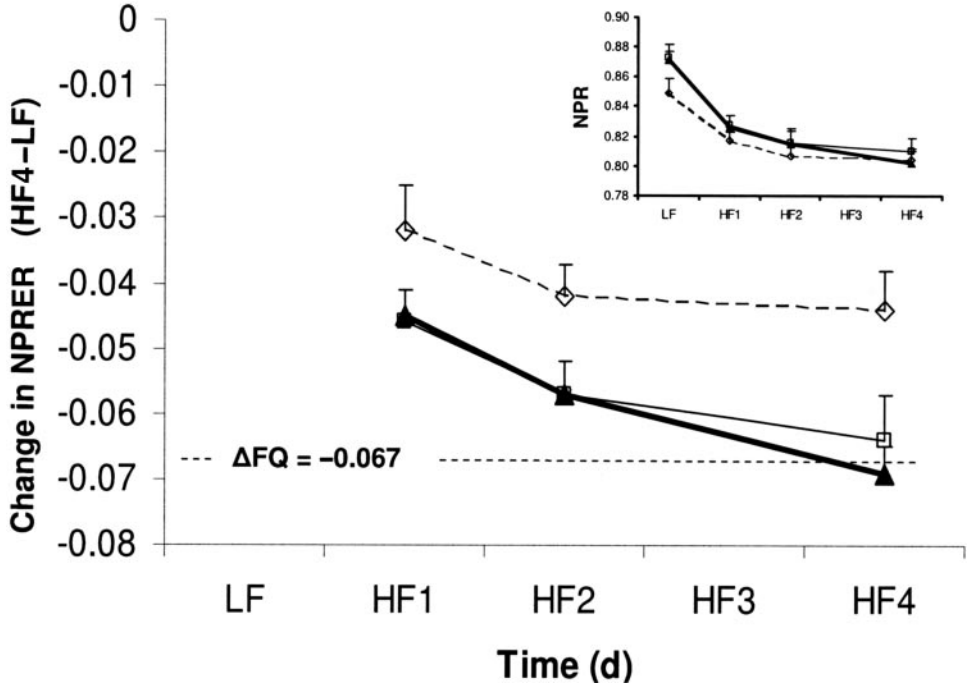

FIGURE 2. Mean (±SEM) change in nonprotein respiratory exchange ratio (NPRER) from baseline during periods of sedentary activity (SED; ◇), 1 h exercise/d (EX1; □), and 2 h exercise/d (EX2; ▲) during consumption of a low-fat (LF) and a high-fat (HF) diet (inset) and change in NPRER from individual baseline values during the LF diet (main panel). HF1–4, days 1–4 of the HF diet. Inset: repeated-measures ANOVA mixed model showed a main effect of time ($P = 0.001$) and an exercise × time interaction ($P = 0.023$). Repeated-measures ANOVA mixed procedure was performed for all data shown. Main panel: A second analysis was performed by using the time-course of NPRER difference from baseline for the 4 HF days. Repeated-measures ANOVA mixed procedure was performed for changes in NPRER for HF data only. Baseline was not included in this statistical model because of collinearity between baseline values and exercise. ΔFQ, the change in the food quotients of the diets provided (HF − LF respiratory quotients). Repeated-measures ANOVA main effects: exercise, $P < 0.001$; time, $P = 0.002$. No significant exercise × time interaction was found.

(1.56 ± 0.02) to EX2 (1.72 ± 0.02), all of which were slightly less than the PALs we had predicted for this experimental design.

Energy balance

Despite the experimental design, average energy balance differed significantly ($P = 0.02$) between exercise treatments. SED, EX1, and EX2 treatments resulted in progressively positive 24-h energy balances averaging 113 ± 21, 164 ± 22, and 200 ± 33 kcal/d, respectively. Post hoc testing identified significant differences in energy balance only between the SED and EX2 treatments ($P = 0.02$). No significant main effect of time ($P = 0.090$) or exercise × time interaction ($P = 0.93$) was seen. Relative to the respective 24-h TEE, energy balances were 5%, 7%, and 8% for SED, EX1, and EX2, respectively.

Respiratory exchange ratio

The RER averaged 0.855 ± 0.006, 0.881 ± 0.009, and 0.878 ± 0.004 for SED, EX1, and EX2, respectively, with the LF diet (day 1 of data collection), compared with the LF diet's food quotient of 0.893. A one-way analysis of variance identified a significant ($P = 0.015$) treatment difference at this baseline time point, which made it an inappropriate baseline from which to begin the analysis. Instead, baseline (LF) was used as a covariate in the statistical model to account for this difference. Protein oxidation was calculated from urinary nitrogen and used to calculate NPRER. NPRER was individually regressed on energy balance (slope: 0.00005; $P < 0.001$), and all 24-h NPRER values were adjusted for energy balance by using this slope.

The 24-h NPRER (**Figure 2**inset) showed a significant decrease with and time ($P < 0.001$) and a time × exercise interaction ($P = 0.023$). Further testing indicates that LF diet day 24-h NPRER for EX1 and EX2 each differed significantly from those for SED ($P = 0.001$ and $P = 0.01$, respectively) when compared individually to SED. We also investigated the change in NPRER on the HF diet days (ΔNPR; Figure 2) that resulted from the subtraction of individual NPRER values for baseline (LF). Statistical analysis showed a significant decrease with increasing exercise ($P < 0.0001$) and a main effect of time ($P = 0.0002$). No significant exercise × time interaction was found ($P = 0.84$). Further analysis again found significant differences between EX1 and EX2 when compared with SED ($P = 0.0002$ and $P < 0.0001$, respectively).

The change in the calculated food quotient (ΔFQ) was −0.067, decreasing from that in the LF diet (0.893) to that in the HF diet (0.826) (Figure 2). Only EX2 ΔNPRER (−0.069) was able to match or exceed the ΔFQ (−0.067), whereas SED and EX1 resolved to −0.44 and −0.063, respectively, after 4 d of the HF diet.

The ΔNPRER main effect of exercise did not differ between the exercise treatments. However, when individual ΔNPRER at HF4 was plotted against measured PAL ($y = -0.07\times + 0.05$), a significant relation was observed ($P = 0.003$), as shown in **Figure 3**.

Fat oxidation

Fat oxidation increased significantly after the switch to the HF diet (**Figure 4**). Fat oxidation shows an interaction between time and exercise ($P = 0.006$). The analysis also found a main effect

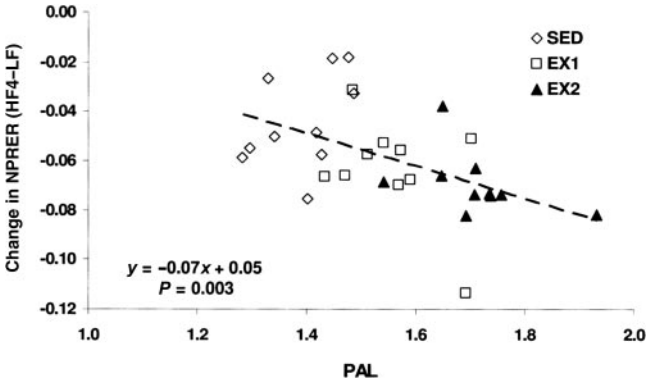

FIGURE 3. Mean physical activity levels (PALs) plotted against mean changes in nonprotein respiratory exchange ratio (NPRER) between the low-fat (LF) diet and day 4 of the high-fat diet (HF4) for individual treatments: sedentary (SED), 1 h exercise/d (EX1), and 2 h exercise/d (EX2). A random-effects model used to analyze the relation between the change in NPRER and PAL resulted in a regression line with a slope different from zero.

of exercise ($P < 0.0001$) and time ($P < 0.0001$). Across-treatment differences are shown in Figure 4 and are most distinct between EX2 and SED.

The greatest increases in fat oxidation between consecutive measures were seen with the transition from the LF diet to HF1 (decreasing the ratio of dietary carbohydrate to lipids) in all treatments (HF1−LF; SED = 18 ± 11 g/d, EX1 = 35 ± 28 g/d, EX2 = 36 ± 1 g/d). Moreover, this increase in fat oxidation was nearly twice as great for EX1 and EX2 as it was for SED. Thereafter, the daily increases in fat oxidation are more gradual: the cumulative increases in fat oxidation between HF1 and HF4 are 8 g for SED, 12 g for EX1, and 21 g for EX2. Indeed, the 4-d cumulative increases in fat oxidation between HF1 and HF4 are less than the 1-d increase between LF and HF1.

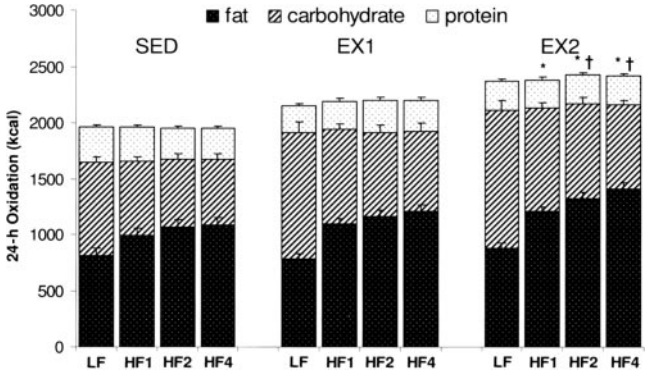

FIGURE 4. Mean (±SEM) 24-h macronutrient oxidation during sedentary activity (SED), 1 h exercise/d (EX1), and 2 h exercise/d (EX2). All symbols shown are for fat oxidation only. *Time point–specific differences from SED for 24-h fat oxidation, $P < 0.05$. †Time point–specific differences from EX1 for 24-h fat oxidation, $P < 0.05$. Student's paired *t* tests for time point differences, $P < 0.05$. The exercise × time interaction for fat oxidation was significant, $P < 0.001$ (repeated-measures model). A repeated-measures ANOVA for carbohydrate oxidation resulted in no significant interaction effect ($P = 0.74$) but significant effects of exercise ($P < 0.0001$) and time ($P < 0.0001$). A repeated-measures ANOVA found no effects of exercise on protein oxidation.

DISCUSSION

In the current study, we found that low-intensity exercise enhances the rates at which NPRER decreases and fat oxidation increases when women switch to an HF diet. This is similar to the results found by Smith et al (21) in men and extends the results to healthy weight and overweight women. We also attempted to extend the findings of Smith et al by testing 2 volumes of low-intensity exercise in each participant. In our study design, we selected the target PAL values of 1.6 and 1.8 because our 2 previous longitudinal studies of weight gain in women suggested a PAL threshold of ≈1.7–1.8, above which there was protection against weight gain (26). Initial analysis of ΔNPRER and fat oxidation data did not detect significant dose effects for the 2 levels of 24-h TEE and hence PAL. Two secondary analyses detected an effect of increasing PAL and a dose-related response. In the secondary analysis, we investigated the relation between individual PAL and ΔNPRER. This regression line showed a distinct and significant relation of greater fat oxidation (ie, lower ΔNPRER) when PAL increases ($P = 0.003$). Data analysis also revealed a similar effect of individual PAL on increased fat oxidation.

We suspect that the failure of the a priori analysis by prescribed PAL to display a difference in ΔNPRER between the PALs of 1.6 and 1.8 may be due to the variances seen in the activities of daily living within the chamber, which blur the exact PAL distinction between treatments. This suggests that the cumulative effects of all physical activities, as summed in PAL, are more important in relation to fat oxidation than in relation to the energy expended in the moderate bicycle activity. This is consistent with our previous findings with bouts of cycling exercise of different intensities—but isocaloric. Moderate and heavy exercise increased dietary fat oxidation after exercise significantly above the level seen in the sedentary condition, and light exercise showed a trend toward an increase (23).

Analysis of energy balance found that participants in the current study were in a constant positive energy balance in all treatments (SED = 113 ± 20; EX1 = 164 ± 21; EX2 = 200 ± 33 kcal/d). This positive energy balance was very comparable to that found by Smith et al (21), in whose study participants were also in positive energy balance throughout the 2 treatments (SED = ≈225; 1.8× RMR = ≈155 kcal/d). Whereas the energy imbalances reported for each treatment in the current study were not by zero as we had designed and are larger than the average daily energy imbalance that would lead to an annual weight gain of 1 to 2 kg, positive energy balance is required for long-term weight gain. The observed small positive energy balances, therefore, may actually be a more useful laboratory model of the periodic episodes that most persons experience in real life (27).

One unexpected observation in our study was that mean NPRER observed with the LF diet were significantly lower [SED = 0.855 ± 0.019 ($P = 0.0001$); EX1 = 0.881 ± 0.028 ($P = 0.21$); EX2 = 0.878 ± 0.012 ($P = 0.003$)] than the FQ of the diet (0.893), and thus we calculated that the participants were in negative fat balance on that day. We cannot explain this observation, but, for 3 reasons, we believe that this baseline variation is not a result of systematic physiologic errors. First, all participants were instructed to abstain from high-intensity exercise for 48 h and from any exercise for 24 h before admission to GCRC, and they were interviewed on admission about activity; second, packaged lead-in diets with 30% of energy from fat were given

to participants for consumption and recording for ≈84 h before GCRC admission; and, third, the 3 treatment orders were randomized.

This study was not designed to investigate the mechanism driving the changes in fat oxidation; however, several mechanisms may be working to produce the effects of diet and exercise that we observed. Changes in the trafficking of dietary fat toward metabolically active tissues, such as skeletal muscle, could be one possible mechanism, and another may be the activity of key enzymes of fat oxidation in those tissues.

The effects of HF diets and exercise are similar in that they both increase the capacity for fat oxidation (28). The gate keeper for dietary fat uptake by tissue is lipoprotein lipase (LPL). Trafficking of dietary lipids is, therefore, highly dependent on tissue-specific LPL activity (muscle, *m*LPL; and adipose, *a*LPL) and these are known to be affected by both diet and exercise (29–32).

In addition to the changes in LPL, there are studies showing that, during (33–35) and after exercise (32, 36), transient increases are seen in the transcription and mRNA of fatty acid translocase/CD36, hormone-sensitive lipase, LPL, and CPT1. Nevertheless, transient increases in the transcription of target genes do not necessarily translate into a one-for-one increase in protein mass. It may take days to weeks for this effect to be fully expressed as increases in these specific proteins and thus to affect clinical outcomes (37). Thus, in our study, the gene expression mechanisms in muscle are most likely to explain the magnitude of the fat oxidation responses accumulating through the end of the 4-d HF diet period, not the short-term response seen between the LF diet day and HF1.

A more likely candidate to explain the observed, rapid, step-like change from LF to HF1 is the regulation of substrate selection (carbohydrate or fat), as influenced by the inhibition or stimulation of the pyruvate dehydrogenase complex (PDC). The regulation of this critical step in substrate oxidation is the gatekeeper for substrate selection and is influenced by pyruvate dehydrogenase kinase (PDK) phosphorylation (38). Considerable evidence suggests that substrate availability drives pyruvate dehydrogenase and PDK activity (33–35, 38–41). Substrate availability is influenced by diet composition (40, 41) and exercise (38). Although gene expression and the resulting enzyme masses can change rapidly, particularly in the liver, it seems more likely that transcription regulation accounts for the changes we observed between HF1 and HF4. In contrast, the 1-d increase in fat oxidation observed between the LF diet day and HF1 is likely to be due to changes in pyruvate dehydrogenase and PDK activities, rather than to an increase in protein mass within lipid oxidation pathways.

In conclusion, these results have 2 practical interpretations. Generally speaking, exercise enhances the ability to increase 24-h fat oxidation in response to increasing intakes of fat in the diet, and, thus, exercise may help in reducing positive fat balance and ultimately weight gain. Moreover, the correlation between PAL and ΔNPRER indicates that the effect of greater fat utilization is enhanced by a greater volume of exercise, which increases the EE. In addition, others suggested that these short-term exposures to HF diets and the small positive fat balances probably accumulate over time to produce excess body fat (27). Thus, regular exercise should be protective in such an environment and should help in the maintenance of a healthy body composition and also provide multiple other health benefits, including protection from cardiovascular disease (42).

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DAS and TG were responsible for the experimental design, and AKA reviewed the design; KCH was responsible for collecting most of the data, and TG assisted in data collection; KCH was responsible for the data analysis; ZZ and KCH were responsible for the statistical analysis; AKA was responsible for medical evaluations; and KCH was responsible for writing the draft of the manuscript, and all authors reviewed and edited the manuscript. None of the authors had a personal or financial conflict of interest.

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